II.

THE POLLEN GRAIN.

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(WITH PLATES XXXV–XXXVI)

It is not my purpose to treat this subject in any detail, but merely to note a few of the more essential and critical points. Thanks are due to Professor Coulter for his criticism, and to many advanced students in the laboratory for the privilege of examining several hundred preparations.

HISTORICAL.

During the past thirty years the embryo sac of spermatophytes has received large attention, and its main structures have been figured and described in many species, but the pollen grains, which are of equal importance, have received but scant attention. The most important literature has been furnished by Hartig, Elfving, Dixon, Guignard, Farmer, and Belajeff. Hartig was the first to describe two nuclei in the ripe pollen grain. Strasburger (1884) greatly extended the researches and described the pollen grains of a great variety of species representing the principal groups of angiosperms and gymnosperms. He showed that the smaller of the two cells in the ripe spore is the generative, also that the generative nucleus undergoes division, giving rise to two male nuclei. This division usually takes place in the pollen tube, but in many cases it takes place in the spore, so that the mature spore may contain three nuclei. Elfving saw three male nuclei in the mature spore of *Andropogon campestris*. Strasburger (1884) reports the occurrence of four male nuclei in the pollen tubes of Ornithogalum and Scilla Guignard (1891) says that in *Lilium Martagon* the division of the generative nucleus occurs only in the pollen tube, and that the tube nucleus never divides at all. Strasburger (1884) also makes the general statement that the tube nucleus never divides.

As long ago as 1884 Strasburger discovered that with a

fuchsin-iodine-green mixture the generative nuclei of pollen grains stain green, and the tube nuclei red; but more recently (1892) he has discussed quite thoroughly the staining reactions of the nuclei. The nuclei of the small prothallial cells of gymnosperm microspores are cyanophilous, like generative nuclei. The nuclei of the nucellus surrounding the embryo sac are also cyanophilous. His conclusion is that the cyanophilous condition in both cases is due to poor nutrition of the nuclei, the amount of cytoplasm being small in proportion to the size of the nuclei. On the other hand, the erythrophilous condition of the nuclei of the embryo sac is due to abundant nutrition. As a further proof of the theory it is noted that the nuclei of the adventitious embryos which come from the nucellus of *Funkia ovata* are decidedly erythrophilous, while the nucellus to which they owe their food supply has cyanophilous nuclei.

In division stages nuclei are cyanophilous. From anaphase to resting stage cytoplasm is taken into the nucleus and the cyanophilous condition gradually changes to the erythrophilous, but when a nucleus is prevented from taking nutrition from a large amount of cytoplasm, as is the case with generative nuclei of pollen grains and the nuclei of the small prothallial cells of gymnosperms, the reaction remains cyanophilous. It is an added proof, that in Ephedra the tube nucleus, which has very little cytoplasm about it, is cyanophilous. Strasburger claims that there is no essential difference between the male and female generative nuclei, and observation shows that within the oosphere spermatozoids and other male generative nuclei become erythrophilous, so that the sex nuclei are alike in their reaction to stains. Malfatti and Lilienfeld have proved that these reactions are dependent upon the amount of nucleic acid. Chromosomes during mitosis consist of nearly pure nucleic acid and are intensely cyanophilous; while cytoplasm has little or no nucleic acid and is erythrophilous. There are all gradations between the cyanophilous and erythrophilous conditions, the affinity for basic anilines being in proportion to the amount of nucleic acid.

It is unfortunate that the terms cyanophilous and erythrophilous are becoming established, since the affinity is for basic or acid dyes, and not for blue or red colors. That the terms are misnomers becomes evident when a combination like safranin (basic) and acid green (acid) is used, for the cyanophilous structures take the red, and the erythrophilous the green.

THE MOTHER CELLS AND TETRADS.

Fairly complete series were obtained in *L. tigrinum* and *L. Philadelphicum*, but since the two species showed very similar results only *L. tigrinum* is described.

The mother cell develops its tetrads after the usual manner among monocotyledons. It was my original purpose to make a cytological study of these cells, chiefly with reference to the phenomena involved in the "reduction division," but my attention was diverted to certain structures of the mature spore, which will be hereafter described. However, certain cytological notes obtained may be of interest.

The nuclei of the mother cells in early spirem stages show a single much twisted ribbon with a row of chromatin granules on each edge. In many cases it could be seen that the chromatin granules were arranged in opposite pairs (*figs. 1 and 1a*). These pairs are separated by a longer stretch of ribbon than is figured by Guignard in his description of *L. Martagon*. The granules are usually more or less ellipsoidal in shape, the longer axis coinciding with that of the ribbon. With cyanin and erythrosin the ribbon stains red, and the granules blue. The ribbon splits longitudinally throughout its entire length before it segments into chromosomes (*fig. 1*). The nuclei showed twelve segments of this double thread in all cases in which the number was definitely ascertained. The further history of the chromosomes and the formation of the spindle were not followed.

In the tetrad stage the nuclear thread is not nearly so intricate, and is often spirally wound inside the nucleus, somewhat like a chromatophore of Spirogyra (*fig. 2.*) In many cases it seemed as if even in spirem stages the position of the future

spindle could be predicted. Centrospheres were observed both in the mother cell and tetrad stages.

THE MATURE SPORE.

The microspore usually reaches its full size and the exine acquires its characteristic markings before its nucleus divides. During the growth which precedes this division the nucleus remains approximately in the center of the cell, but just before the division it often moves toward one end of the spore (*fig.* 4). The position of the nuclei, however, often indicates that division has taken place without any such preliminary movement (*fig.* 5). Nearly all the mature microspores of *L. tigrinum* present essentially the conditions represented in *figs.* 5 and 6. The tube nucleus is larger and erythrophilous, while the generative nucleus is cyanophilous. The number and size of nucleoli vary, but as a rule the nucleoli of the tube nucleus are larger than those of the generative nucleus. The chromatin network of the tube nucleus is much finer and more irregular than that of the generative nucleus.

Such cases as *figs.* 6–8 are common, and they give the impression that a wall is separating the generative and tube cells. When the generative cell is lenticular and pressed against the wall of the spore it is usually at one end (*fig.* 6), but occasionally it is at one side (*fig.* 7). The cytoplasm of the generative cell was entirely free from starch except in one instance.

Centrospheres were observed in connection with both the tube and generative nuclei. It is comparatively easy to demonstrate centrospheres with the generative nucleus on account of the uniformity in their position and the small amount of cytoplasm in the generative cell. The tube cell is richly supplied with starch, which differs greatly in appearance as different stains are used.

The foregoing applies to most of the pollen grains of the species studied, but occasionally an anther was found which showed very different conditions. The most common variation was the division of the generative nucleus while still within the

pollen grain (*figs. 8, 9*), a condition not uncommon in monocotyledons. More than a hundred such cases were noted in *L. tigrinum*, and about thirty in *L. auratum*, but they are rare in *L. Philadelphicum*. In two cases in *L. auratum* a further division of the generative nucleus was observed, resulting in three male cells (*fig. 10*).

There is abundant negative testimony for the usual statement that the tube nucleus never divides, but hundreds of pollen grains of *L. tigrinum* and *L. auratum* presented such division. In *fig. 12* there are four nuclei, all of which have the characteristics of tube nuclei. One pollen grain was found with eight nuclei, six of which were vegetative and two generative (*fig. 15*). Numerous cases like *figs. 3, 13* and *14* prove that this division is of the direct or amitotic type.

It may be noted in this connection that the cells of the tapetum often contain two, three, or even four nuclei which have been produced by the direct process. No evidence of mitosis was observed in the cells of the tapetum or in connection with the tube nucleus, but it is possible that it occurs in both cases, and that these nuclei may divide by either process. The significance of amitosis seems to be little understood in either animals or plants. The frequency of the phenomenon in pathological tissues has led to the theory that it is due to degeneration. On the other hand, such cases as the internodes of the Characeae suggest that it aids metabolism by increasing the nuclear surface. Its occurrence in gland cells connects it with extreme cell activity. If all cases of amitosis are to have the same explanation it must be much more inclusive than any of these suggested. In Lilium a single tube nucleus seems to suffice in the vast majority of cases. If amitosis is a degenerate condition from mitosis, the division of the tube nucleus might have a phylogenetic significance.

The pollen grains of *L. tigrinum* often showed another variation which seems to be quite important. In *fig. 17*, in addition to the tube and generative nuclei, there is shown a small cell cut off from the end of the spore. A similar condition is seen in

fig. 16, but here the generative cell has effected its ordinary divisions. Over twenty such cases were observed. I have called the cell nuclei marked *g* (*figs. 16, 18*) male nuclei, that is, daughter nuclei from the generative nucleus, because their nucleoli are small, their chromatin network coarse, and in staining they are cyanophilous, that is, they show a preference for basic dyes. Besides, some of these nuclei are surrounded by definite areas of cytoplasm devoid of starch, indicating the organization of the male cell. The tube nucleus, or the several nuclei to which it may give rise, has larger nucleoli, finer chromatin network, and is uniformly erythrophilous. Hence it seems safe to conclude that the nuclei marked *g* in *figs. 16, 18* are generative in origin, and not vegetative like the tube nucleus and its derivatives. The small cell marked *pr* (*figs. 16, 18*) is hard to interpret. Its nucleus is cyanophilous, and its cytoplasm is free from starch. In these respects it resembles the generative nucleus and its derivatives, and if the tube nucleus were the only other nucleus in the spore I should call the small cell a much reduced generative cell. However, a study of all the cases discovered leads me to suggest that the small cell is a prothallial cell, homologous with the single prothallial cell of heterosporous pteridophytes. The small cell cut off from the microspore of *Populus monilifera*, figured but not described in my paper on Salix, adds probability to this hypothesis. If this interpretation is correct, it supports the view that the whole spore development, as it ordinarily appears, is an antheridium. In this case the tube nucleus and its cytoplasm is probably the homologue of the wall cells of such an antheridium as that of Isoetes; the pollen tube would become an outgrowth from the antheridium wall; and the two male cells would homologize with the spermatozoid mother cells. At least it seems out of the question to speak of the pollen tube as the male gametophyte.

Another peculiar phenomenon was noted in *L. tigrinum*. In about twenty cases there was a distinct wall dividing the microspore into two nearly equal parts (*figs. 19-20*). Both cells contained starch, and when each cell contained but one nucleus they

stained alike. In *fig. 20* one of the cells contains two nuclei which seem to represent generative and tube nuclei, and two other such cases were observed. In these cases, also, I am inclined to regard one of the cells as prothallial, and the other antheridial.

EXPLANATION OF PLATES XXXV–XXXVI.

All figures, except *fig. 1 a*, were drawn with an Abbe camera lucida, $\frac{1}{12}$ Bausch and Lomb immersion, and Zeiss ocular 4. The combination gives a magnification of 1010 diameters. *Fig. 1 a* was drawn with $\frac{1}{12}$ Bausch and Lomb, ocular 18 Zeiss. The drawings are reduced one-half by photography: *g*, generative nucleus, *pr*, prothallial cell. *Figs. 3, 9, 10* are from *L. auratum*, all others from *L. tigrinum*.

FIG. 1. Mother cell of pollen grain; the ribbon splitting longitudinally.

FIG. 1 *a*. Small portion of ribbon showing usual shape and position of chromatin granules. Cyanin and erythrosin.

FIG. 2. Young pollen grain from tetrad, showing centrosomes and spiral arrangement of ribbon.

FIG. 3. Mature pollen grain with two tube nuclei and two generative nuclei; one of the tube nuclei suggests direct division.

FIG. 4. Division of primary nucleus of the pollen grain.

FIG. 5. Generative nucleus accompanied by centrosomes; starch quite conspicuous.

FIG. 6. Very common position of generative cell.

FIG. 7. Less common position of generative cell.

FIG. 8. Generative nucleus divided.

FIG. 9. Generative nuclei divided; one generative nucleus and the tube nucleus accompanied by centrosomes.

FIG. 10. Three generative nuclei.

FIG. 11. Tube nucleus divided.

FIG. 12. Four nuclei, all with characters of tube nuclei.

FIG. 13. Three nuclei, two of which indicate direct division.

FIG. 14. Two tube nuclei and one generative nucleus; one of the tube nuclei shows direct division.

FIG. 15. Six tube nuclei and two generative nuclei.

FIG. 16. One tube nucleus, two generative nuclei, and a prothallial cell.

FIGS. 17–18. One tube nucleus, one generative nucleus, and a prothallial cell.

FIG. 19. A definite wall separating the spore into two approximately equal parts.

FIG. 20. Same as preceding, but one part showing what may be interpreted as a generative nucleus and a tube nucleus.

III.

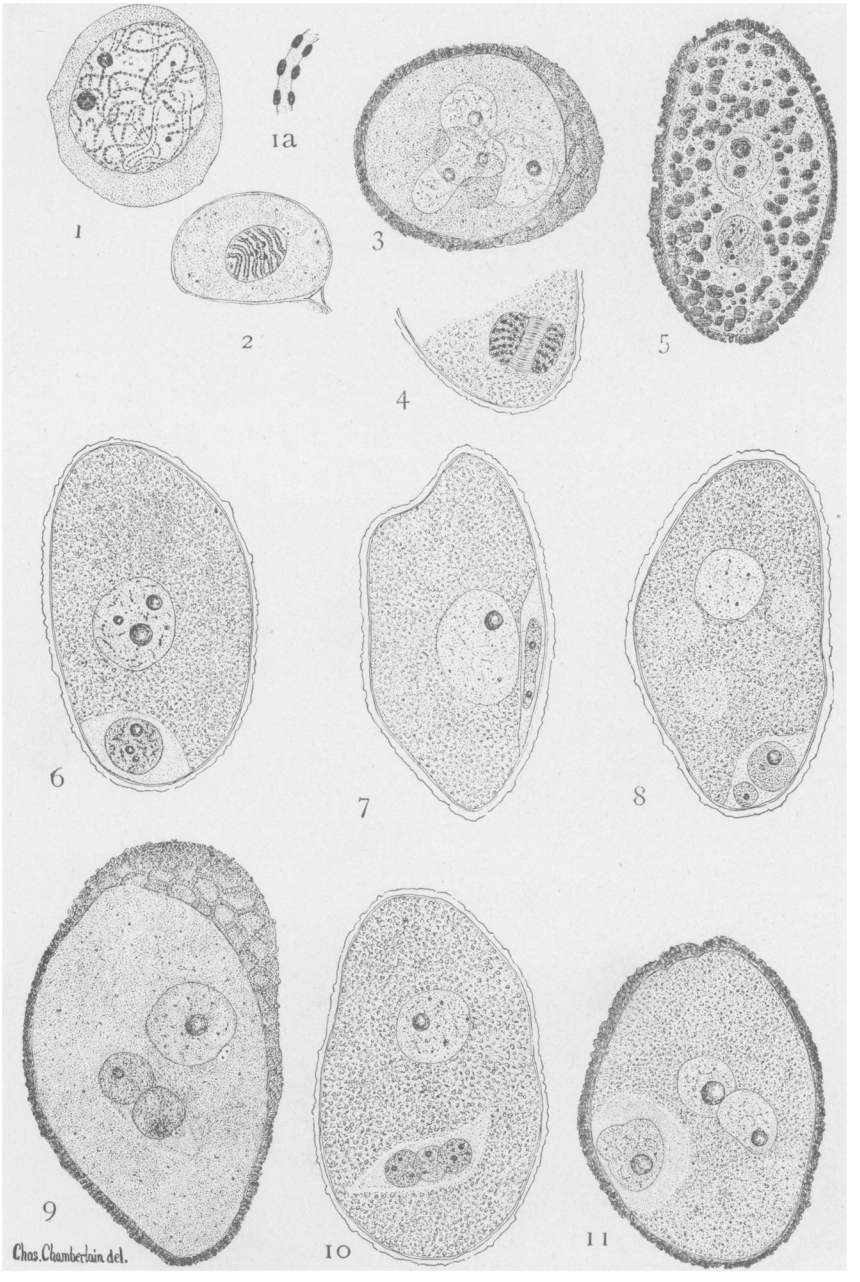
THE DIVISION OF THE MACROSPORE NUCLEUS.

JOHN H. SCHAFFNER.

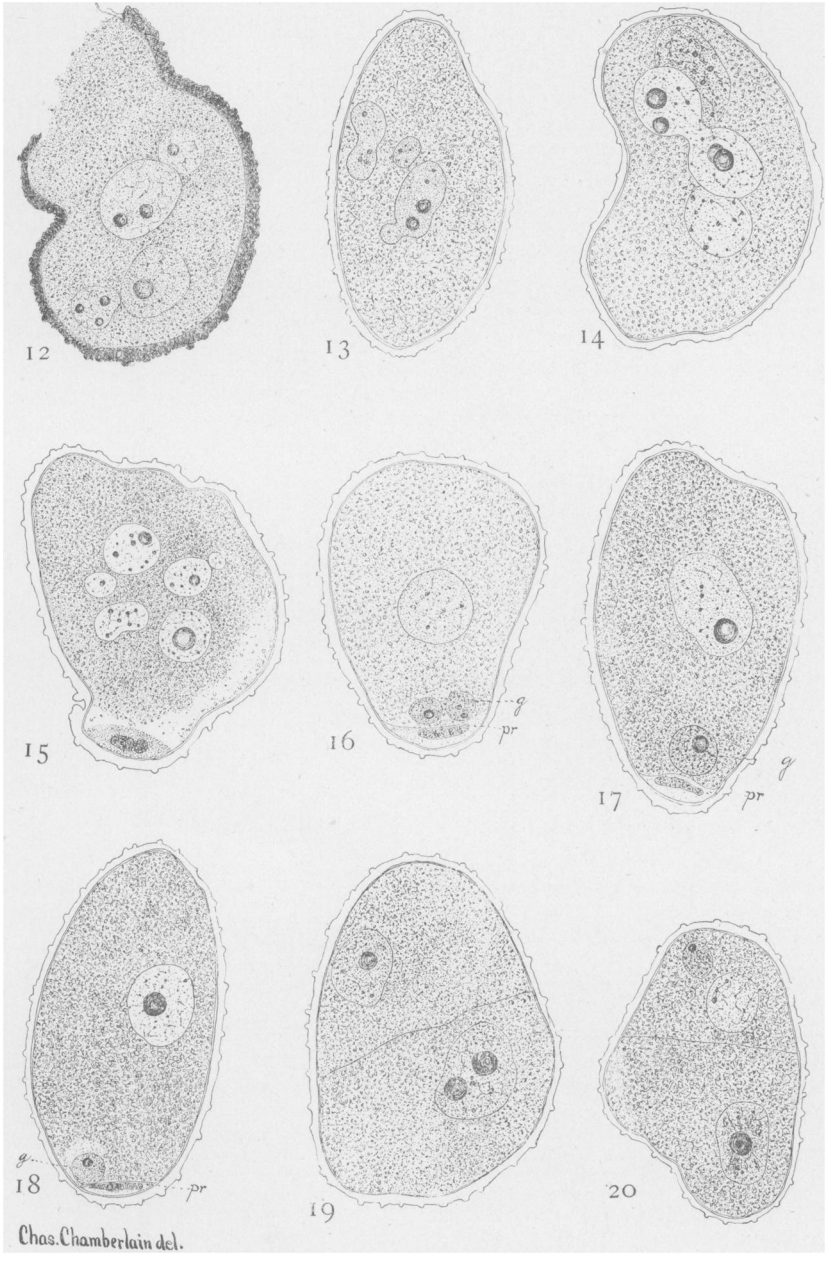
(WITH PLATES XXXVII–XXXIX)

Although a knowledge of the changes which take place in the reduction nuclei of plants and animals is of the utmost importance, and will no doubt aid more than anything else in bringing about a correct interpretation of the facts of heredity, comparatively little has been done in this field, and the observations that have been reported disagree widely. This may be accounted for because of the extreme difficulty of properly preparing suitable material for study, and of correct observation and interpretation of the minute structures concerned. The following work was undertaken because especially favorable material was at hand, and some peculiar variations from what has been received as the normal process of reduction were observed. During the course of the investigation the writer was compelled several times to abandon preconceived notions obtained from the literature of the subject. Whatever, therefore, is presented in regard to the formation of chromosomes and the activities of the nucleoli during karyokinesis has not been the outcome of an attempt to establish evidence which would be agreeable to some hypothesis, but the whole investigation presented an array of facts conclusive to the writer's mind.

My thanks are due to Dr. John M. Coulter for his interest and supervision, as well as to a considerable number of coworkers in the laboratory who kindly permitted me to study and compare their preparations with my own.



CHAMBERLAIN on LILIUM.



CHAMBERLAIN on LILIUM.